Thermogenetic curves and thermokinetics of seed germination of *Robinia pseudoacaia*

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Abstract: Seed germination process has closely relation with material transformation and energy exchange within the seed. Study on its thermal effect is important for understanding the mechanism and the influencing factors of the seed germination. The thermogenetic curves of seed germination of *Robinia pseudoacacia* was measured by a new-type conductive microcalorimeter made in Wuhan University. The relationship was analyzed between the germination thermogenetic regulation and seed germination physiology. The thermogentic curves were further analyzed by thermokinetic theory to obtain the dynamic parameters and the thermokinetic model on seed germination of *Robinia pseudoacacia*. The relationship of the thermogenetic power(μ w) and the germination time(h) of the germination process of 20 grains *Robinia pseudocacia* seeds at 25℃ was P=208.77/[0.1937+0.8063exp(-0.06563t)]

Key words: thermogenetic curve; thermokinetics; seed germination; *Robinia pseudoacacia*

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Introduction

The seed shape had a great change from germination to sprout, which was based on the exchange of the material within the seed. This change was a process of the decomposition of amylum and fats and the differentiation and propagation of cell in an embryo or a cotyledon. Meantime, a certain heat effect must be produced during those material transformation processes. If it can be continually measured by a calorimeter with enough sensibility, the thermogentic curves may be obtained. This provides a new suitable method for the evaluation of seed germination characteristic. The further analysis for the thermogenetic curves is helpful to deeply understand the mechanism and influencing factor of seed germination. Prat and Calvet had measured the germination thermogram of wheat seed. (Part and Calvet 1944). Microcalorimetric method has been applied widely to life sciences now (Lamprecht 1985; Lamprecht *et al.* 1991; Criddle *et al.* 1991).

Robinia pseudboacaia Linn is very good tree specie to the water-soil conservancy, sand-break and wind-break, environmental afforestation in the west-north and north China. The germination regulation of *Robinia pseudoacacia* seed adapted to study with microcalorimetic method because its germination time is short.

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Materials and methods

Materials

Robinia pseudoacacia seeds in this experiment were provided by Hubei Tree Seed Company.

Instrumentation

The experiment was carried out by a new-type microcalorimeter made in Wuhan University. It is a heat conductive microcalorimeter with twin microcalorimetric cells. The thermopile comprises 603 pairs of thermoelements in each cell. The microcalorimetric system comprises a main part of the microcalorimeter, a thermostat, an electric energy standard and a recorder. The main part of the microcalorimeter is shown in Fig.1. Its basic principle is that many pairs of thermoelements are situated between the calorimetric cell and block. When the heat produced from the sample moving to the metal block through the thermopile, the temperature difference and the thermoelectricity were recorded by the recorder. If there is no further heat output, the curve drawn by the recorder will come back to base line as the temperatures between the sample and the metal block tend to equilibrium. The heat power and heat quantity can be obtained according to the height and peak area of thermogram.

Principal performance indexes of this microcalorimetric system are that the precision of thermostat control temperature system is $< \pm 0.001$ K, the effective volume of microcalorimetric cell is 25 mL, sensitivity is about 3 μ w, determinable precision is $< \pm 1\%$, and the time constant is about 277 s (Wan *et al.* 1994).

This microcalorimeter has the advantage of high precision temperature control, large and effective cell volume, high detection sensitivity, simple construction and easy operation etc. The microcalorimeter is particularly suitable for the monitoring and studying biological processes, as many biological processes have characteristics of small heat effect and slow change process.

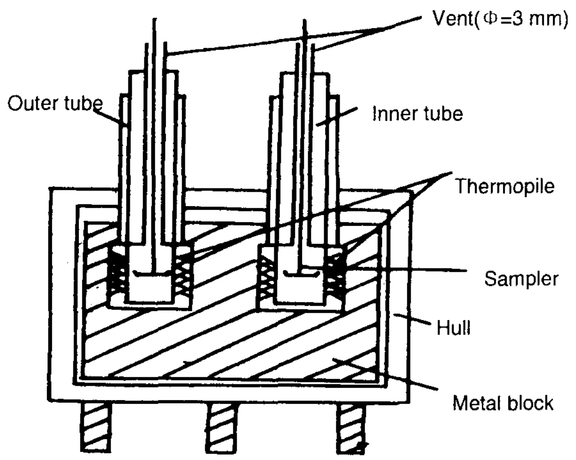

Fig.1 Sketch of the main part of microcalorimeter

Methods

The germination experiments of *Lobinia pseudoacacia* seeds were carried out at 25℃ in darkness. Three-mL distilled water was respectively put in the measuring cell and the reference cell of the microcalorimeter. Twenty grains of seeds (about 0.5 g) were set on the sample injector in the measuring cell, and the system temperature was kept equilibration about 10 h. Then the sample injector with the seeds in the measuring cell and the empty sample injector in the reference cell of the microcalorimeter were slowly put in contact with 3 mL of distilled water in both cells at the same time. Thermogenetic curves of seed germination were recorded continuously by the recorder for about 80 h. Electric calibrations were made at the end of the run. And in the same conditions the control experiment was done to observe the shape variation of the seeds.

Results and discussion

Thermogenetic curves of seed germination

The seed germination experiments of *Robinia pseudoacacia* were done and the germination thermogrenetic curves was obtained at 25℃ (shown in Fig.2).

In Fig.2 the ordinate is the thermogenetic power, stands for the heat-product quantity in unit time during the germination of *Robinia pseudoacaia* seeds, the abscissa is the experiment time. On the thermogenetic curve of the germination of *Robinia pseudoacacia* seeds, there is a sharp heat liberation peak in 10 min soon after an endothermic peak, and then the seeds go back into heat liberation stage. Before 30 h the thermogenetic power is lower, but after 30 h, the thermogenetic power shows speedily rise. After 26 h the thermogenetic curve is S type.

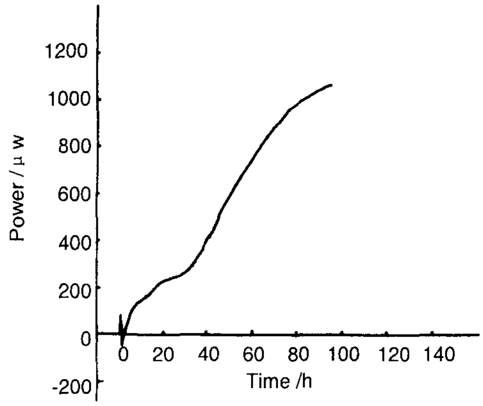

Fig.2 Thermogenesis curve of seed germination of *Robinia pseudoacacia* at 25℃

By comparing the thermogenetic curve with the shape variation, water absorption and breath process, the seed germination process of *Robinia pseudoacacia* could be discussed with three stages.

1) Hydration stage (0-5 h): in this stage, there exist two different actions. One is physicochemical thermogenesis (Prat and Calvet 1944). The wetting heat is released because of the water absorption and wetting action in 10 min after the seeds contacted with water. Another is endothermic action that is caused by the water absorption and swelling of testa and germ.

2) Active stage (5-26 h): after hydration, the prepared enzymes begin activation, and the respiration intensity of seeds has a rising trend. With energy metabolism, and thermogenetic strengthening, the thermogram shows a quickly rising trend. In the later of active stage, the water uptake in cotyledon has saturated and the expanded testa limits oxygenic supply, so seed transforms normal breath into anaerobic respiration. Meantime, the water absorption and breath cease, and then thermogram relatively steady. In active stage, the stored material of seed is decomposed and transformed by the active enzyme, and complex insoluble amylum, grease, and protein are decomposed into simple available soluble glucose and ammo acid. With the decomposition and the transformation of nutrient material, seed still keeps the energy metabolism and thermogenesis. The active stage was called biochemical thermogenetic stage too (Trione and Cony 1990).

3) Growth stage (after 26 h): After germination point the embryonic root breaks through the teata to improve the oxygenic provision of the seed. The embryonic root grows quickly. New chondriosome and the respiratory enzyme are synthesized, and the seed shows strong water uptake and breath again. With the ordered growth of embryonic root and embryonic bud, the nutrient materials in seed are transformed quickly and thermogenetic power increased fast. After a certain time growth, as the cell quantity of embryonic root and embryonic bud quickly increasing, the

limiting factors such as transporting resistance of the nutrient material and darkness are getting strengthened, so the growth of embryonic bud was gradually limited. Therefore the thermogrom of the growth stage shows S type, that is, the thermogenetic process changes from relatively steady to quickly rising, then gradually comes into steady.

Thermokinetics for growth stage of seed germination

As the thermogram of the growth stage of seed germination in *Robinia pseudoacacia* is S type, so it can be analyzed by Logistic equation (Thornly 1975). In the process of seed germination, the cell cleavage has two cases. On the one hand, the cell cleavage speeds up with the cell quantity increase, on the other hand, as the number of cell increased, some factor of limiting cell cleavage intensified, such as the cell quantity fast increasing, the transporting resistance of the nutrient material strengthening, and the dark overtime and so on, the cell cleavage is limited. So the speed equation of the cell clearage is

$$dN/dt = kN(1 - sN) = kN - skN^2 \tag{1}$$

where *k* stands for the constant of growth speed, *s* stands for the limiting factor of the limited growth. If $\beta = sk$, stands for decay speed constant. If the heat of the cell metabolism is direct ratio with the cell quantity, then the heat power measured in the experiment is

$P = P_0 N$

Its differential is

$$dP/dt = P_0 dN/dt$$

Substituting it into equation (1), we have

$$dP/dt = kP(1 - sP/P_0) = k(P - sP^2/P_0)$$

Its integral is

$$\ln[P(1-s)/(P_0 - sP)] = kt \tag{2}$$

Taking regulate of equation (2), we have

$$\ln[(P_0/s)/P - 1] = \ln[(1-s)/s] - kt$$

if $\alpha = P_0/s$ then

$$\ln(\alpha/P - 1) = \ln[(1-s)/s] - kt \tag{3}$$

Taking regulate of equation (2), we can obtain the thermokinetic equation of the seeds germination.

$$P = P_0/[s + (1-s)]\exp(-kt) \tag{4}$$

Substituting the heat power *P* and the growth time *t* obtained from the thermogenetic curve of the germination of *Robinia pseudoacacia* seeds into equation (3), choosing the suitable α value to make best linearity, we can get the growth rate constant *k*, the limiting factor *s* and specific power P_0. The test value of *Robinia pseudoacacia* seeds and the calculation results are listed Table 1 and Table 2.

Table 1. The heat power (*P*) and growth time (*t*)of growth stage

T/h	6	12	18	24	30	36	42
P/μw	275	365	502	588	678	766	853
T/h	48	54	60	66	72	78	
P/μw	917	963	1001	1049	1095	1149	

Table 2. The thermokinetic parameters of the growth stage

$k\times10^2$/h^{-1}	$s\times10^2$	$\beta\times10^2$/h^{-1}	P_0/μw	r
4.69	20.718	0.9717	260.01	-0.995

According to the parameters Table 1 and Table 2, we can write the thermokinetic equation of the growth stage of the germination of *Robinia pseudoacacia* seeds at 25℃,

$$P = 260.01/[0.20718 + 0.79282\exp(-0.0469t)].$$

Conclusion

The thermogenetic curves of seed germination of *Robinia pseudoacacia* that was determined by the microcalorimetic method could reveal the correlations between the law of producing heat and the laws of water absorption, breath, and growth. Meanwhile we could obtain the thermokinetic parameters of seed germination of *Robinia pseudoacacia* in growth stage, and further build the thermokinetic model of the growth stage for seed germination of *Robinia pseudoacacia*. These results have an important meaning for studying the seed germination mechanism and effect factors, and for determining seed strain and seed vigor. It has opened up a new way for the study of the germination mechanism of other tree seeds in other suitable place.

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